

Aspirin Sensitivity and IgE Antibodies to *Staphylococcus aureus* Enterotoxins in Nasal Polyposis: Studies on the Relationship

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Key Words

Aspirin sensitivity · Nasal polyposis · *Staphylococcus aureus* enterotoxins · ECP · IgE · Interleukin-5

Abstract

Background: Nasal polyposis is a multifactorial disease characterized by a chronic eosinophilic inflammation of the sinus mucosa, often associated with asthma and aspirin sensitivity. We have recently shown that the presence of IgE antibodies to *Staphylococcus aureus* enterotoxins (SAEs) was related to the severity of eosinophilic inflammation in nasal polyp tissue. In this study, we therefore aimed to determine, whether aspirin sensitivity was related to an immune response to SAEs, and how both criteria would be related to eosinophilic inflammation. **Methods:** 40 subjects with nasal polyposis (NP) were classified as aspirin-sensitive (n = 13, ASNP) or aspirin-tolerant (n = 27, ATNP) based on a bronchial aspirin challenge test. Homogenates prepared from nasal polyp tissue and inferior nasal turbinates from healthy subjects (n = 12) were analyzed for concentrations of IL-5 by enzyme immunoassay and for ECP, total and IgE to a mix of SAEs (A, C, TSST-1) using the ImmunoCAP system. **Results:** Concentrations of IL-5, ECP, total IgE, and IgE to an SAE mix were significantly increased in ASNP compared with ATNP patients and controls. In addition, a

subgroup analysis showed an increase in eosinophilic markers in ATNP-SAE(+) compared to ATNP-SAE(–). This relationship, however, was not found in ATNP-SAE(+) and ATNP-SAE(–) subjects, indicating that SAE immune response is overlapped or not relevant in this condition. **Conclusions:** Aspirin sensitivity was associated with increased concentrations of eosinophil-related mediators, as well as IgE antibodies to SAEs in nasal polyp tissue. However, a direct impact of *S. aureus* could not be established. It seems that aspirin sensitivity and immune reactions to SAEs are independently related to eosinophilic inflammation.

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Introduction

Nasal polyposis (NP) is a multifactorial disease characterized by chronic eosinophilic inflammation of the upper airway mucosa, i.e. the nose and sinuses [1]. This pathology is often associated with asthma and aspirin sensitivity and both T helper 1 (Th1) or 2 (Th2) type cytokines are upregulated in tissue independent of the atopic status [2]. Sensitivity of the airways to acetylsalicylic acid (aspirin) is characterized by polypous rhinosinusitis, severe, often steroid-dependent asthma and adverse airway reactions upon ingestion of aspirin or other nonsteroidal anti-inflammato-

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ry drugs (NSAIDs) [3]. The disease pattern in aspirin-sensitive patients is characterized by chronic inflammation of the upper and lower airways, often accompanied by an increase in blood and tissue eosinophils and elevated numbers of T lymphocytes, macrophages and mast cells in bronchial mucosa, nasal secretions and serum [4, 5]. Of interest, patients with aspirin-sensitive NP (ASNP) frequently show more severe disease and local eosinophilic inflammation compared with nonsensitive or aspirin-tolerant (ATNP) NP subjects, as well as an increased rate of recurrence after treatment including surgery [6, 7].

In addition, abnormalities in arachidonic acid metabolism have been found in ASNP patients characterized by a significant difference in cyclooxygenase/lipoxygenase product ratios compared with normal nasal mucosa [8]. Also, a low production of PGE₂, which downregulates the cyclooxygenase-2, and over-expression of LTC₄ synthase resulting in increased release of cys-LTs [9, 10] have been demonstrated in these patients.

Bacterial infection may also be implicated in chronic eosinophilic inflammatory disorders [11]. Superantigens, especially *Staphylococcus aureus* (*S. aureus*) enterotoxins (SAEs) have been demonstrated to potentially influence the pathogenesis of chronic skin and respiratory diseases, such as dermatitis, perennial allergic rhinitis and NP [12–14].

Clinical and experimental studies indicate that nasal polyp formation and growth are initiated and perpetuated by an integrated process involving the mucosal epithelium, extracellular matrix, and inflammatory cells, which in turn may be triggered by both infectious and noninfectious inflammation [15]. In a recent study, we discussed the possible role of SAEs in NP. We demonstrated that the presence of IgE antibodies to SAE A/SAE B correlated to the severity of eosinophilic inflammation in terms of eosinophilic cationic protein (ECP), interleukin-5 (IL-5) and eotaxin protein levels [16]. In this study, we therefore aimed to determine whether clinically documented aspirin sensitivity was related to an immune response to SAEs, and how both criteria would be related to eosinophilic inflammation.

Material and Methods

Patients

For this study, polyp tissue was obtained from 40 patients with bilateral nasal polyps, who were scheduled for sinus surgery. The diagnosis of NP was based on a typical history and symptoms of NP such as nasal congestion, rhinorrhea and loss of smell, and confirmed by nasal endoscopy prior to surgery. The diagnosis of aspirin sensitivity was made on the basis of a positive bronchial aspirin provocation

Table 1. Characteristics of the patient groups used in the study

	Control	ATNP	ASNP
Subjects	12	27	13
Age, years	39 ± 13.4	57 ± 15	52 ± 13.7
Male/female	6/6	17/10	4/9
Inhaled steroids	0	100	100
IgE-SAE (+)	0	7	7
IgE-SAE (–)	12	20	6
Allergic patients	0	3	10
Asthmatic patients	0	6	13

test. In the NP group, 13 individuals showed sensitivity to aspirin and 27 were not sensitive to this drug (table 1). All of the aspirin-sensitive subjects also suffered from asthma, and had a history of at least one bronchial reaction to nonsteroidal anti-inflammatory drugs (NSAIDs).

A skin prick test for a panel of inhalant allergens including house dust mites, grass and tree pollen, cat dander and feathers was performed. 13 out of 40 NP patients had at least one positive test, with 10 of these patients belonging to the aspirin-sensitive NP group (ASNP) (table 1). As control group, we included 12 subjects, who underwent septal surgery and removal of parts of the inferior turbinate due to anatomical variations. None of these subjects had a history of atopic disease, NP, asthma or adverse reactions to NSAIDs. The ethics committee of Medical University of Lodz approved the study and all patients gave informed consent before their participation. The use of any medication related to the disease, including glucocorticoids and anti-leukotrienes was stopped in all subjects 4 weeks before surgery.

Asthma and aspirin sensitivity highly coincided in our patient population, and it was impossible to differentiate the two clinical parameters. It should be noted that, as we selected the patients on the basis of aspirin sensitivity, aspirin-sensitive asthmatics are overrepresented in comparison to aspirin-tolerant subjects, and no conclusions can be deduced for asthmatics in general.

Aspirin Challenge Protocol

Lysine-acetylsalicylic acid (lysine-ASA; Aspisol, Horby Bayer AG, Germany) was administered with a dosimeter-controlled jet nebulizer (Spira Electro 2, Respiratory Care Center, Hameelinna, Finland). The lysine-ASA challenge test was only performed if the patient's FEV₁ was ≥ 60% of predicted on the day of challenge. Three fresh stock solutions of 2 M, 1 M solution and 0.1 M of lysine-ASA were prepared and used within 4 h of preparation. The solution in the nebulizer chamber was kept at 12 °C between administrations of doses, but brought to room temperature before each administration. The nebulizer delivered an aerosol with 80% of particles being less than 5.8 µm, and a median mass diameter of 4.1 µm. The use of 3 different concentrations of lysine-ASA solution and varying the number of tidal breaths (1–23) resulted in stepwise increments in the dose of inhaled lysine-ASA. Baseline FEV₁ was measured as the best of three efforts before (pre-diluent baseline) and 20 min after the inhalation of 7 breaths of 0.9% sodium chloride (post-diluent baseline). If the post-diluent FEV₁ was <90% of the pre-diluent FEV₁, the lysine-ASA challenge was not performed due to instability of the bronchial tree. The post-diluent baseline was used to calculate the percent drop

Table 2. Concentrations of inflammatory markers and IgE in NP, ASNP, ATNP and control groups (median + interquartile ranges)

	IL-5, pg/ml	ECP, µg/l	IgE to SAEs, kU/l	Total IgE, kU/l
NP	108.14 (22–231)	3.0 (1.5–8.8)	1.9 (2–7)	164.1 (50–502)
ASNP	274.6 (127–667)	8.7 (3.0–14.1)	10.2 (2–20)	472.7 (296–706)
ATNP	57 (22–127)	2.4 (1.2–6.6)	1.93 (1.9–3)	82.1 (35–266)
Controls	21.4 (21.3–21.5)	51.4 (17–1.3)	0.5 (0.5–0.5)	14.9 (0.5–32)

in FEV₁ caused by lysine-ASA, which was inhaled in half-log incremental doses every 30 min. FEV₁ was obtained at 10, 20 and 30 min after the last inhalation of each dose. The lysine challenge was completed when FEV₁ decreased by ≥20%, compared to the post-diluent baseline or the maximum dose of ASA had been reached (540 µmol cumulated dose). The patient was observed for at least 1 h after the termination of the provocation, and if necessary, 2 puffs of salbutamol were administered.

Tissue Collection and Processing

Tissue samples were collected during sinus or septal surgery and immediately frozen in liquid nitrogen and stored at –70°C. 100 mg of tissue was homogenized in 1 ml of 0.9% NaCl solution (mechanical homogenizer B. Braun, Melsungen, Germany) at 1,000 rpm for 5 min on ice [17, 18]. After homogenization, the suspensions were centrifuged at 3,000 rpm, 4°C for 10 min to collect supernatants for ELISA. All prepared samples were stored at –70°C until analyzed.

Quantification of IL-5 and ECP in Tissue Homogenates

IL-5 was measured by an Enzyme Immunoassay (EIA) purchased from R&D Systems (Minneapolis, USA). The assay has a detection range between 7.8 and 500 pg/ml and a sensitivity of <3 pg/ml. Amounts of ECP and total IgE in supernatants obtained after homogenization of nasal polyp tissue were quantified by a UniCAP system (Pharmacia & Upjohn, Sweden), with a detection limit of <0.5 µg/l for ECP and 0.35 kU/l for total IgE measurements.

Quantification of Total IgE and Specific IgE in Tissue Homogenates

To screen for IgE antibodies to SAEs in nasal polyp samples, we established a mix of three SAEs (SAE A, SAEC and TSST-1) coupled to a solid-phase (ImmunoCAP- system, Pharmacia Sweden, cut-off 0.35 kU_A/l). No nonspecific IgE reactivity to the ImmunoCAP SAE mix was found for non-antibody active IgE (E myeloma) at concentrations up to 1,000 kU/l. Samples positive for IgE antibody to the SAE mix tested negative to a control ImmunoCAP without allergen.

Statistical Analysis

Data are expressed as median and interquartile ranges. The Mann-Whitney U test was applied using MedCalc version 6.0 to evaluate the statistical differences between groups. The Kruskal-Wallis test (H test) was applied to compare the data within the different patient subgroups. To evaluate statistical significance of differences between two parameters, Spearman's rank correlation analysis was applied. The χ^2 test was used to test the statistical significance of differences between different classification groups. A p value of 0.05 or less was considered as statistically significant.

Results

Eosinophil-Related Mediators and IgE in NP versus Controls

A significant increase in IL-5 concentrations ($p < 0.01$) was observed in samples from supernatants in NP patients compared to controls. IL-5 strongly correlated with ECP ($r = 0.70$, $p < 0.01$), which was also significantly upregulated ($p < 0.01$) in the NP group. A significantly higher concentration of total IgE and IgE antibodies to SAEs in nasal polyp tissue was observed compared to healthy subjects ($p < 0.01$). In addition, levels of IgE to SAEs correlated with the IL-5 ($r = 0.37$, $p = 0.02$) and ECP ($r = 0.49$, $p = 0.002$) levels in the NP group. Data are summarized in table 2.

Eosinophil-Related Mediators and IgE in ASNP and ATNP Patients

NP patients were further analyzed according to two classification groups, with or without aspirin sensitivity (ASNP and ATNP, respectively). Results are summarized in figure 1 and table 2. Quantities of IL-5 and ECP were upregulated in ASNP and differed significantly from ATNP and control subjects. Concentrations of total IgE and IgE antibodies to a mix of SAEs (SAE A, SAE C, TSST-1) showed significantly higher levels in ASNP patients compared to ATNP and control groups as well.

Eosinophil-Related Mediators and IgE in ASNP (ATNP) – IgE to SAE Subgroups

A post hoc analysis, considering aspirin sensitivity as the main classification criterion, was performed in order to investigate whether the immune response to SAEs (expressed in levels of IgE antibodies to SAEs) was related to the inflammatory reactions and changes in arachidonic acid metabolites in NP patients. ASNP and ATNP patients were each divided in two subgroups: SAE(+) and SAE(–).

Patients with levels of IgE antibodies to SAEs above 0.35 kU_A/l were included in the group we assume had pre-

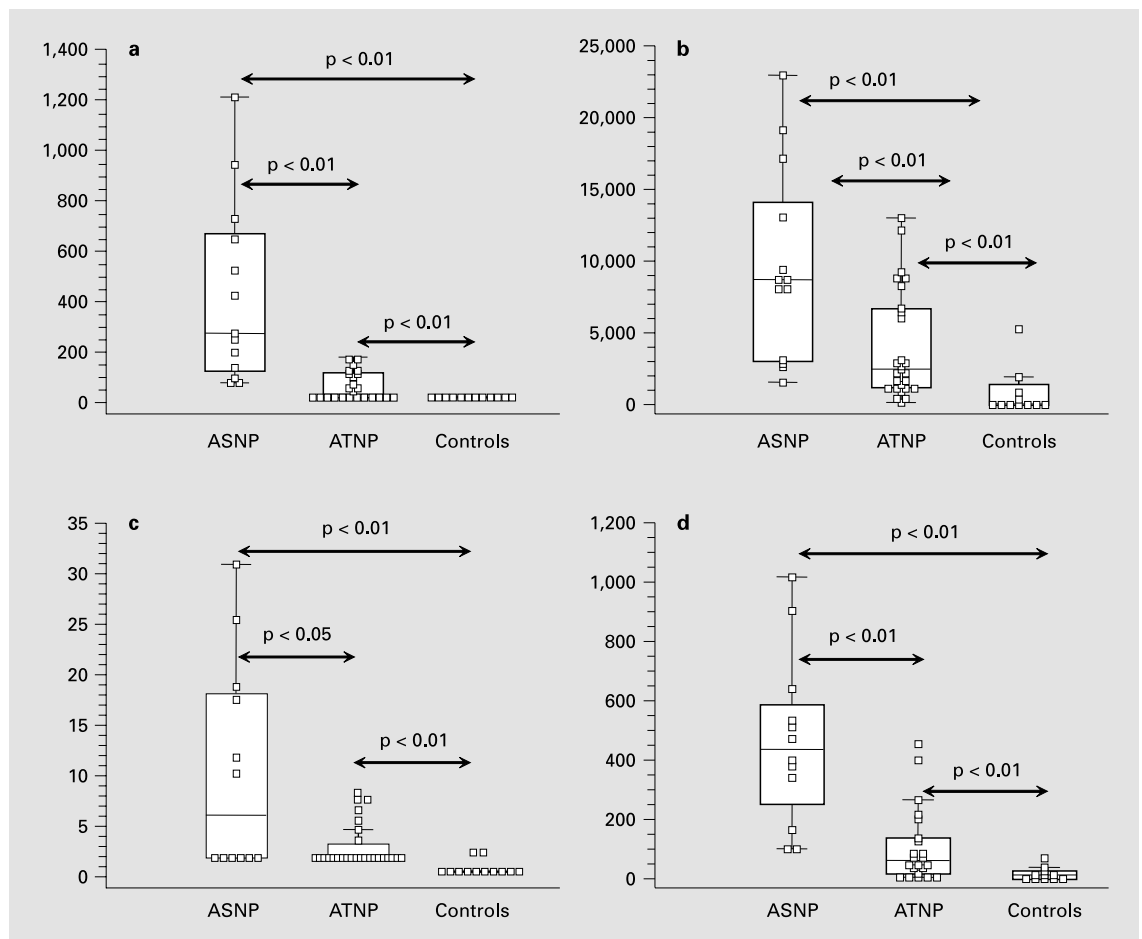


Fig. 1. Concentrations of IL-5 (pg/ml) (**a**), ECP ($\mu\text{g/l}$) (**b**), specific IgE antibodies (kU_A/l) (**c**) and total IgE antibodies (kU_A/l) (**d**) to SAEs, in nasal polyp tissue from ASNP ($n = 13$), ATNP ($n = 27$) and in nasal turbinate tissue from the control group ($n = 12$). The box and whiskers plot represents the median, the lower to upper quartile, and the minimum to the maximum value, excluding outside and far out values. Statistical analyses were performed by using the Mann-Whitney U test.

vious or current exposure to SAEs [SAE(+)]. Subjects containing no IgE antibodies or concentrations of less than $0.35 \text{ kU}_A/\text{l}$, were considered as SAE negative [(SAE(-)]. Out of 13 patients with ASNP, 7 were SAE(+) (54%) in contrast to 7 out of 27 in the ATNP group (26%) and none out of 12 subjects in the control group (0%, $p = 0.003$).

Data were analyzed by the Kruskal-Wallis test and results are summarized in table 3 and figure 2. Concentrations of inflammatory markers (IL-5 and ECP) did not differ between ASNP-SAE(+) and SAE(-) groups, but were upregulated with respect to the control group. Interestingly, the pattern for ATNP groups was different, as IL-5 and ECP levels were significantly increased in the SAE(+) compared to SAE(-) patients and controls.

Discussion

In this study, we have demonstrated that concentrations of eosinophil-related markers such as IL-5 and ECP, as well as total IgE and IgE antibodies to SAEs in polyp tissue were significantly increased in ASNP compared with ATNP patients and controls. A post hoc analysis did not reveal any direct link between SAEs and inflammatory reactions in ASNP. However, in ATNP, the immune response to these enterotoxins was linked to the severity of eosinophilic inflammation.

We have previously demonstrated that IgE antibodies to SAEs in NP patients were related to the severity of eosinophilic inflammation, and possibly to the clinical

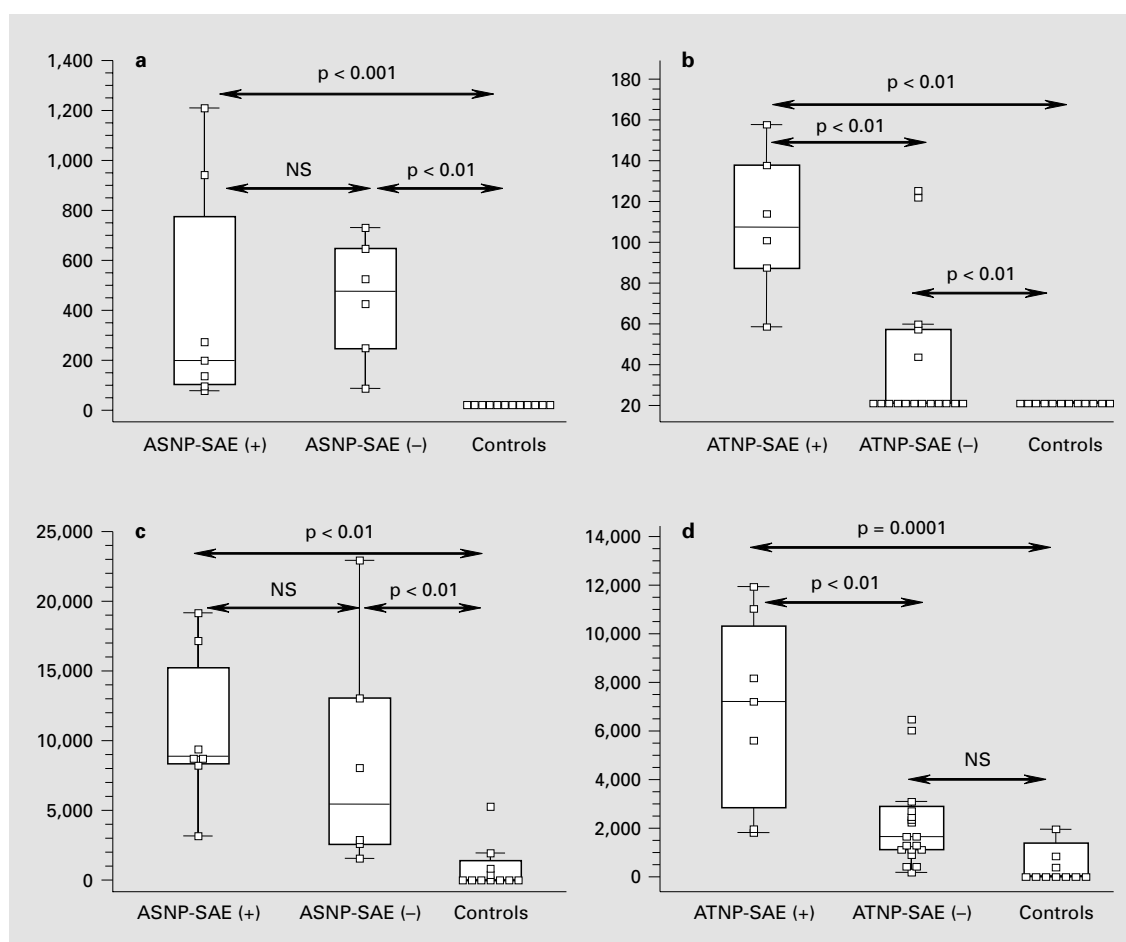


Fig. 2. Concentrations of IL-5 (pg/ml) (**a, b**) and ECP (µg/l) (**c, d**) in nasal polyp tissue homogenates and nasal turbinates from ASNP-SAE(+), ASNP-SAE(-), control subjects and ATNP-SAE(+), ATNP-SAE(-) and control subjects. The box-and-whiskers plot represents the median, the lower to upper quartile, and the minimum to the maximum value, excluding outside and far out values. Statistical analyses were performed using the Mann-Whitney U test.

Table 3. Concentrations of inflammatory markers and IgE in ASNP, ATNP-SAEs subgroups (median + interquartile ranges)

	IL-5, pg/ml	ECP, µg/l	IgE to SAE, kU _A /l	Total IgE, kU/l
ASNP-SAE (+)	198.8 (106–775)	8.8 (8.3–152.2)	18.8 (13–29)	472.7 (383–838)
ASNP-SAE (-)	475.2 (250–647)	5.4 (2.6–13.0)	1.9 (1.9–1.9)	426.5 (107–533)
ATNP-SAE (+)	128.2 (105–167)	8.3 (3.9–11.4)	6.6 (4.9–7.9)	787.6 (201–2,270)
ATNP-SAE (-)	21.6 (21–57)	1.6 (1.1–2.9)	1.9 (1.9–1.9)	50.8 (15–105)

manifestation of asthma [16]. Fleischer [19] has shown that staphylococcal enterotoxins can act as superantigens by stimulating accessory or target T cells by cross-linking the variable part of the beta chain of the T cell receptor with MHC class II molecules. This leads to a multiclonal stimulation of up to 20–25% of the naive T-cell population in a nonspecific way, and also induces a multiclonal IgE synthesis and severe eosinophilic inflammation [16]. In addition, SAEs has been demonstrated to inhibit eosinophil apoptosis and modulate cell surface markers on these cells [20] and to induce the production of Th2-type cytokines such as IL-5 [13].

In the present study, we were able to confirm former results [17] showing significant upregulation of IL-5 protein in samples from NP tissue homogenates versus controls, which showed a firm correlation to ECP in NP samples. Also confirming earlier results [16], concentrations of total and IgE antibodies to SAE mix (A, C and TSST-1) were significantly higher in NP tissue homogenate compared to control nasal mucosa, and IgE antibodies to SAEs correlated with the concentrations of ECP and IL-5 in the NP group. We now show that concentrations of IL-5 and ECP were especially increased in ASNP subjects

compared with controls and/or ATNP patients, in line with earlier work [7]. We furthermore show that concentrations of total IgE antibodies and of IgE antibodies to SAEs were significantly increased in ASNP subjects compared to controls and ATNP patients. 54% of ASNP patients demonstrated IgE antibody production against SAEs, compared to 26% in the ATNP and none in the control group, which reflects a significant difference between these groups. Finally, data from a subgroup analysis of ASNP and ATNP for the criterium 'IgE antibodies to SAEs' showed that severity of inflammation is related to the presence of IgE to SAE in the group of ATNP patients only. The increase in the levels of inflammatory mediators in the ATNP-SAE(+) compared to SAE(–) but not in ASNP-SAE(+) compared to SAE(–) patients reveals that immune response to SAEs represents a disease modifier unrelated to aspirin sensitivity.

Aspirin sensitivity was associated with increased concentrations of eosinophil-related mediators, as well as IgE antibodies to SAEs in NP tissue. However, a direct impact of *S. aureus* on NP could not be found. It seems that aspirin sensitivity and immune reactions to SAEs are independently related to eosinophilic inflammation.

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